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Molecular Cytogenetic Diagnosis of Williams Syndrome

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Williams syndrome (WS) is characterized by distinct facial changes, growth deficiency, mental retardation, and congenital heart defect (particularly supravalvular aortic stenosis), associated at times with infantile hypercalcemia. Molecular genetic studies have indicated that hemizygosity at the elastin locus (7q11.23) causes WS. The purpose of this study was to confirm that this regional deletion, involving the elastin locus, is the cause of WS in Japan, and to clarify the correlation between the phenotype and the elastin locus.

Thirty-two patients with WS and thirty of their relatives were examined by fluorescent in situ hybridization (FISH), using the WS chromosome region (WSCR) probe. All patients had cardiovascular disease (100%), 30 had typical WS facial changes (94%), 31 had mental retardation or developmental delay (97%), 16 were small-for-date at birth (50%), 14 had short stature (44%), and 13 had dental anomalies (41%). No relatives showed any manifestation of WS.

Hemizygosity for a region of 7q11.23, involving the elastin locus, was found in all WS patients, but was not found in the 30 relatives. © 1996 Wiley-Liss, Inc.

KEY WORDS: Williams syndrome, fluorescent in situ hybridization, hemizygosity of elastin gene, Williams syndrome chromosome region probe, congenital heart disease

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INTRODUCTION

Williams syndrome (WS) is a developmental disorder characterized by a unique facial phenotype, mental retardation, short stature, and characteristic personality. Congenital heart defect (particularly supravalvular aortic stenosis) is often present and infantile hypercalcemia is sometimes seen in this disorder [Williams et al., 1961; Black et al., 1963; Kelly et al., 1975; Beuren, 1972; Burn, 1986]. The syndrome generally occurs sporadically, although familial cases are known and follow an apparent autosomal dominant pattern of inheritance [Cortada et al., 1980; Morris et al., 1993]. Molecular genetic studies have indicated that hemizygosity at the elastin locus (7q11.23), causes WS [Ewart et al., 1993; Keating et al., 1994].

The purpose of this study was to confirm that a regional deletion involving the elastin locus causes WS in Japan, and to clarify the correlation between the phenotype and the elastin locus by fluorescent in situ hybridization (FISH), using the WS chromosome region (WSCR) probe with a D7S427 Chromosome 7 Control probe (Oncor).

MATERIALS AND METHODS

Patients

Thirty-two patients (eleven males and twenty-one females) with Williams syndrome and thirty of their relatives (ten pairs of parents, nine mothers, and one father) were examined in this study (Table I). All patients were sporadic cases and Japanese. Of the 32 patients, 16 were seen at the Heart Institute of Japan, Tokyo Women's Medical College, and 16 were from elsewhere. The cardiac diagnosis was confirmed by echocardiography and angiocardiography. Pressure gradients across the supravalvular aortic stenosis were measured by cardiac catheterization or pulse Doppler echocardiography [Ensing et al., 1989]. The typical manifestations of each patient were diagnosed independently by three cardiologists based on the WS diagnostic criteria of the Preus index [Preus et al., 1984].

Fluorescent In Situ Hybridization

Metaphase human chromosome preparation. Human metaphase chromosome slides were prepared

TABLE I. The Clinical Findings in Patients With Williams Syndrome*

No.	Age	Sex	Face	MR(DD)	Cardiovascular findings	Other findings
1	24Y	F	+	+	SVAS, PPS	Dental anomalies
2	3Y	F	+	+	SVAS, PPS	SFD, dental anomalies
3	3Y	F	+	+	SVAS, PPS	SFD, short stature
4	10M	F	+	+	SVAS, PPS, PH	SFD, short stature
5	8Y	F	+	+	SVAS, PPS, VSD, PLSVC, PH	SFD, short stature
6	12Y	F	+	+	SVAS, PPS	SFD, short stature
7	29Y	M	+	+	SVAS, PPS	SFD, short stature, I.hernia, dental anomalies
8	4Y	M	+	+	SVAS, PPS	SFD, short stature, absence of left kidney
9	17Y	F	±	+	SVAS, PPS, Valvular PS	
10	27Y	F	±	+	SVAS, PPS	Dental anomalies, strabismus
11	6Y	F	+	+	SVAS, PPS, VSD, PLSVC	Short stature, dental anomalies
12	7Y	M	+	+	SVAS	Hoarse voice
13	9Y	M	+	+	SVAS, PPS	Hoarse voice, I.hernia, dental anomalies
14	20Y	M	+	+	SVAS, CoA	
15	32Y	F	+	+	SVAS, MVP	I.hernia, dental anomalies
16	25Y	M	+	+	SVAS	Dental anomalies
17	12Y	M	+	+	SVAS, MVP	Dental anomalies, hoarse voice, I.hernia
18	27Y	M	+	+	SVAS	SFD, I.hernia, strabismus
19	11Y	F	+	+	SVAS	Short stature, dental anomalies
20	10Y	F	+	+	SVAS, PLSVC	SFD, short stature, dental anomalies, I.hernia
21	5Y	M	+	+	SVAS, IAA, VSD, PH	Short stature
22	7M	F	+	+	SVAS, PPS	Webbed neck
23	6Y	F	+	+	Valvular PS	Short stature, hoarse voice, strabismus
24	20Y	F	+	+	AVP, MVP	SFD, dental anomalies, hoarse voice
25	5Y	F	+	+	SVAS, Ebstein, PPS	SFD, short stature, hoarse voice
26	18Y	M	+	+	SVAS	Hoarse voice
27	16Y	M	+	+	SVAS, WPW	Hoarse voice
28	8Y	F	+	+	SVAS	SFD, dental anomalies, hoarse voice
29	6Y	F	+	+	SVAS	SFD, short stature
30	1Y	F	+	±	SVAS, valvular PS, PPS	SFD, hoarse voice
31	3Y	F	+	+	SVAS, RVOTO, PPS	SFD, short stature, I.hernia, hoarse voice
32	10Y	F	+	+	SVAS, PPS	SFD, hoarse voice, I.hernia

*AVP, aortic valve prolapse; CoA, coarctation of the aorta; DD, developmental delay; Ebstein, Ebstein anomaly; Face, Williams syndrome facial features; IAA, interruption of the aortic arch; I.hernia, inguinal hernia; MR, mental retardation; MVP, mitral valve prolapse; PH, pulmonary hypertension; PLSVC, persistent left superior vena cava; PS, pulmonary stenosis; PPS, peripheral pulmonary stenosis; RVOTO, right ventricular outflow tract obstruction; SFD, small for date; SVAS, supravalvular aortic stenosis; VSD, ventricular septal defect; WPW, Wolff-Parkinson-White syndrome.

from Epstein-Barr virus-transformed lymphoblastoid cell lines and/or peripheral blood by standard methods [Yoshida et al., 1986]. DNA on the slides was denatured in 70% formamide/ 2 × SSC for 2 minutes at 70°C and immediately dehydrated through a cold (-20°C) ethanol series, and then air-dried.

Fluorescent in situ hybridization and detection. The WSCR probe (Oncor) consisted of two probes containing the 5' and 3' end of the elastin gene, respectively [Ewart et al., 1993]. Figure 1 shows the WSCR and chromosome 7 control probes. The hybridization mixture containing the WSCR probe with a D7S427 Chromosome 7 control probe (Oncor) was placed on denatured chromosome slides for in situ hybridization. After overnight hybridization at 37°C in a moist chamber, the slides were washed once for 10 minutes in 50% formamide/2 × SSC at 37°C, once for 5 minutes in 2 × SSC at room temperature, once in 1 × PBD (Oncor), and once in 1 × PBD/5% BSA. For anti-digoxigenin-Rhodamin detection, the method of Matsuoka et al. [1993, 1994] was employed. Briefly, the slides were incubated with anti-digoxigenin-Rhodamin (Boehringer Mannheim) in 1 × PBD/5% BSA at 37°C for 60 minutes and rinsed in 1 × PBD 3 times for 2 minutes each time. After washing, the slides were stained with DAPI and mounted with antifade solution. For observation of fluorescence signals on the chromosomes, a Carl Zeiss fluorescence microscope equipped with appropriate fluorescence-filter sets were used. For digital fluorescence microscopy, a cooled charged coupled device (CCD) camera (Photometrics) was used. Digital images were processed using software IPLab SpectrumTM (Signal Analytic Corporation). FISH analysis was performed on all Williams syndrome patients and the 30 family members. At least 20 metaphases from each patient and their family members were analyzed.

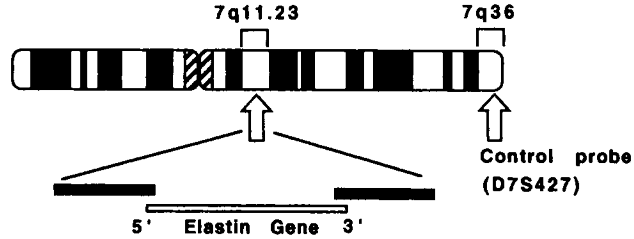

Fig. 1. Bold bars represent the Oncor probes to the Elastin gene region.

RESULTS

Cytogenetic Studies

FISH, using the WSCR probe, was performed on all patients with WS and all 30 of their relatives. Hemizygosity for a region of 7q11.23 involving the elastin locus (Fig. 2) was found in all patients with WS, but not in any of the relatives.

Clinical Findings

The clinical findings of the 32 patients with WS are shown in Table I. All had congenital heart disease (32/32, 100%), 30 had a typical WS face (30/32, 94%) (Fig. 3), 31 had mental retardation or developmental delay (31/32, 97%), 16 were small-for-date at birth (16/32, 50%), 14 had short stature (14/32, 44%), 13 dental anomalies involving malocclusion and microdontia (13/32, 41%), 12 hoarse voice (12/32, 38%), 8 inguinal hernia (8/32, 25%), and 3 strabismus (3/32, 9%).

Of the cardiovascular findings, supravalvular aortic stenosis was most frequently seen (30/32, 94%)(Fig. 4). Pressure gradients across the stenosis ranged from 0 to 110 mm Hg (mean 24 mm Hg). Seventeen of the thirty patients with supravalvular aortic stenosis had peripheral pulmonary arterial stenosis (17/30, 57%). Two of the seventeen patients with peripheral pulmonary arterial stenosis had proximal pulmonary hypertension (2/17, 12%). Ventricular septal defect and mitral valve prolapse were each seen in three patients. Ebstein anomaly, interrupted aortic arch, Wolff-Parkinson-White (WPW) syndrome, and coarctation of the aorta were each seen in one patient. None of the relatives had any sign of WS.

DISCUSSION

Several phenotypes of Williams syndrome may be attributed to hemizygosity at the elastin locus. Elastin is the main protein in the aorta, and supravalvular aortic stenosis is well documented in WS. Pathologic studies of the aortic wall of WS patients with supravalvular aortic stenosis showed disorganized elastic fibers [O'Connor et al., 1985; Perou et al., 1961]. In our data, supravalvular aortic stenosis was most frequently seen (30/32, 94%), and peripheral pulmonary arterial stenosis (17/32, 53%), and coarctation of the artery (1/32, 3%)

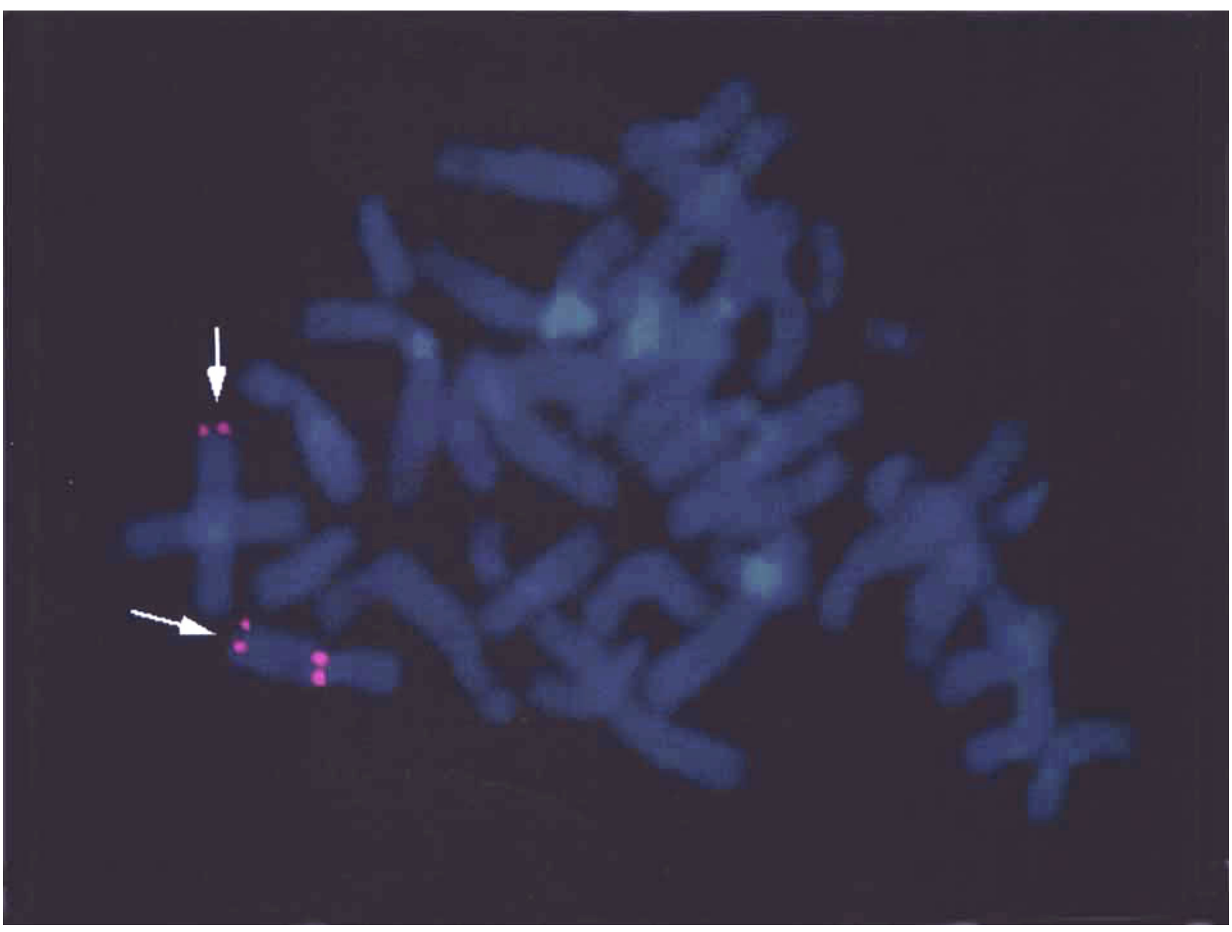

Fig. 2. A metaphase spread from a Williams syndrome patient's (No. 31) hybridized mixture containing the Williams Syndrome Chromosome Region (WSCR) probe with a 7q36 Chromosome 7 Control Probe. The arrows indicate the direction of chromosome 7 (telomere to centromere). Signals can be seen at the 7q36 (chromosome 7 control) and 7q11.23 (WSCR) on the normal chromosome (lower arrow), and only at 7q36 on the deleted chromosome (upper arrow).

Fig. 3. Patient no. 32, a 10-year-old girl with Williams syndrome facial features.

were seen as other types of congenital narrowing of the artery. Furthermore, hoarse voice, some of the WS facial changes, and inguinal hernia may be explained by the presence of abnormal elastin. These abnormalities may be caused by the decreased dose of elastin that results from hemizygosity at the elastin locus.

With regard to the other cardiac findings, ventricular septal defect (three patients), mitral valve prolapse (three patients), Ebstein anomaly (one patient), interrupted aortic arch (one patient), and WPW syndrome (one patient) were seen. To our knowledge, the Ebstein anomaly or interrupted aortic arch or WPW syndrome have not yet been reported in WS. These three cardiovascular disorders, mental retardation, short stature, small-for-date at birth and hypercalcemia are not likely to be explained by hemizygosity at the elastin locus alone. Deletion of the contiguous gene of elastin, or mutation at other loci may also be contributory factors.

The phenotype of WS shows wide variation from individual to individual. Furthermore, as several genetic studies have demonstrated that mutations involving part of an elastin gene cause autosomal dominant supravalvular aortic stenosis [Ewart et al., 1994; Curran et al., 1993; Morris et al., 1993], and familial supravalvular aortic stenosis patients occasionally have mild WS facial changes and hoarse voice as well as WS, we conclude that WS is a contiguous gene deletion syndrome [Nickerson et al., 1995], and the size of the deletion in WS seems to show a wide individual variation.

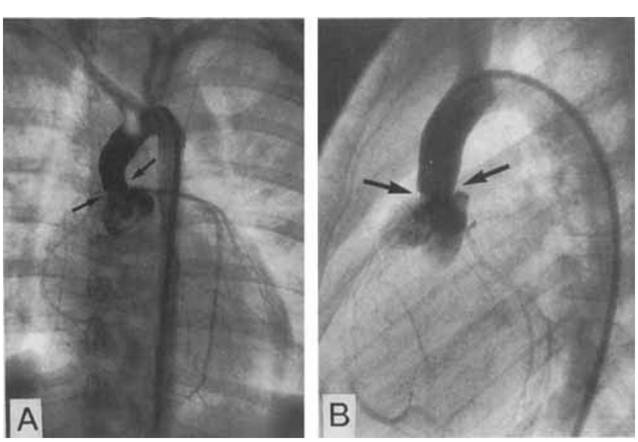

Fig. 4. Angiogram of patient no. 17. The arrows show the supravalvular aortic stenosis. **A,** Frontal view. **B,** Lateral view.

The clinical diagnosis of WS depends on the clinical phenotype. However, occasionally, WS individuals do not have a typical WS face, clear mental retardation, or cardiovascular anomalies [Jones et al., 1975; Morris et al., 1988]. Thus, a clinical diagnosis of WS may be difficult due to the wide variation of phenotypes. Furthermore, WS is often overlooked during infancy, as it is a progressive disorder and clinical phenotypes may not be clear during infancy. Therefore, there is a tendency for a diagnosis to be made late (mean age at diagnosis, 6.4 years) [Morris et al., 1988]. In these patients, it is necessary to confirm the diagnosis of WS by FISH using the WSCR probe. If these cases of WS are confirmed by FISH during the neonatal period, the patients can receive better treatment, including nutrition and therapy for cardiovascular disease and abnormal metabolism. Also, we can give earlier genetic counseling to the families of the patients.

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REFERENCES

Beuren AJ (1972): Supravalvular aortic stenosis: a complex syndrome with and without mental retardation. BD:OAS VIII (5):45–56.

Black JA, Bonham Carter RE (1963): Association between aortic stenosis and facies of severe infantile hypercalcemia. Lancet 2: 745–748.

Burn J (1986): Williams syndrome. J Med Genet 23:389–395.

Cortada X, Taysi K, Hartman AF (1980): Familial Williams syndrome. Clin Genet 18:173–176.

Curran ME, Atkinson DL, Ewart AK, Morris CA, Leppert MF, Keating MT (1993): The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. Cell 73:159–168.

Ensing GJ, Schmidt MA, Hagler DJ, Michels VV, Carter GA, Feldt RH (1989): Spectrum of findings in a family with nonsyndromic autosomal dominant supravalvular aortic stenosis: A Doppler echocardiographic study. J Am Coll Cardiol 13:413–419.

Ewart AK, Jin W, Atkinson D, Morris CA, Keating MT (1994): Supravalvular aortic stenosis associated with a deletion disrupting the elastin gene. J Clin Invest 93:1071–1077.

Ewart AK, Morris CA, Atkinson D, Jin W, Sternes K, Spallone P, Dean Stock A, Leppert M, Keating MT (1993): Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nat Genet 5:11–16.

Jones KL, Smith DW (1975): The Williams elfin facies syndrome. J Pediatr 86:718–723.

Keating M (1994): Elastin and vascular disease. Trends Cardiovasc Med 4:165–169.

Kelly JR, Barr ES (1975): The elfin facies syndrome. Oral Surg 40: 205–218.

Matsuoka R, Yoshida MC, Furutani Y, Imamura S, Kanda N, Yanagisawa M, Masaki T, Takao A (1993): Human smooth muscle myosin heavy chain gene mapped to chromosomal region 16q12. Am J Med Genet 46:61–67.

Matsuoka R, Takao A, Kimura M, Imamura S, Kondo C, Joh-o K, Ikeda K, Nishibatake M, Ando M, Momma K (1994): Confirmation that the conotruncal anomaly face syndrome is associated with a deletion within 22q11.2. Am J Med Genet 53:1–8.

Morris CA, Thomas IT, Greenberg F (1993): Williams syndrome: autosomal dominant inheritance. Am J Med Genet 47:478–481.

Morris CA, Demsey SA, Leonard CO, Dilts C, Blackburn BL (1988): Natural history of Williams syndrome: physical characteristics. J Pediatr 113:318–326.

Morris CA, Loker J, Ensing G, Dean Stock A (1993): Supravalvular aortic stenosis cosegregates with a familial 6;7 translocation which disrupts the elastin gene. Am J Med Genet 46:737–744.

Nickerson E, Greenberg F, Keating M T, McCaskill C, Shaffer LG (1995): Deletions of the elastin gene at 7q11.23 occur in~90% of patients with Williams syndrome. Am J Hum Genet 56:1156–1161.

O'Connor WN., Davis JB, Geissler R, Cottrill CM, Noonan JA, Todd EP (1985): Supravalvular aortic stenosis: clinical and pathologic observations in 6 patients. Arch Pathol lab Med 109:179–185.

Perou M (1961): Congenital supravalvular aortic stenosis. Arch Pathol 71:113–126.

Preus M (1984): The Williams syndrome: objective definition and diagnosis. Clin Genet 25:422–428.

Williams JCP, Barratt-Boyes BG, Lowe JB (1961): Supravalvular aortic stenosis. Circulation 24:1311–1318.

Yoshida MC, Satoh H, Sasaki M, Semba K, Yamaoto T, Toyoshima K (1986) : Regional location of a novel yes-related proto-oncogene, syn, on human chromosome 6 at band q21. Jpn J Cancer Res 77:1059–1061.